DATA EVALUATION REPORT

Acute Oral Toxicity (LD50) Study in Albino Rats with Pine Oil Blend CSMA 1687
MRID 40253502

Study Type: Acute Oral Toxicity
OPPTS 870.1100

Prepared for

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Contract Number: 68-W-01-036
Work Assignment No.: 0248.3000.001.02 TAF 1-2-6
EPA WAM: Killian Swift, Ph.D.

This review may have been altered by EPA subsequent to the contractors' signatures above.

EPA Reviewer: Tim McMahon
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Signature:
Date 1-5-04

Template version 11/01

TXR#: 1003261

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Albino Rat, OPPTS 870.1100 [§81-1]; OECD 401.

PC CODE:

DP BARCODE:
SUBMISSION NO.:

TEST MATERIAL (PURITY): Pine Oil Blend CSMA 1687 (purity not reported)

SYNONYMS: Pine Oil

CITATION: Naas, D. (1987) Acute Oral Toxicity (LD50) Study in Albino Rats with Pine Oil Blend CSMA 1687. WIL Research Laboratories, Inc., Ashland, Ohio. Study number WIL-114001. June 19, 1987. MRID 40253502. Unpublished.

SPONSOR: Chemical Specialities Manufacturers Association, Suite 1120, 101 Connecticut Ave, Washington, DC, 20036.

EXECUTIVE SUMMARY:

In an acute oral toxicity study (MRID 40253502), groups of fasted, young adult Sprague-Dawley rats (25 males and 20 females) were given a single oral dose of undiluted Pine Oil Blend CSMA 1687 (purity and lot number unknown) by intubation. The doses were 2183, 2500, 2863, 3278, and 3753 mg/kg bw in males and 1907, 2183, 2500, and 2863 mg/kg in females. Following dosing, the rats were observed for 14 days.

Oral LD_{50} **Males = 3.1 (95% C.I., 2.7-3.6) g/kg bw**
Females = 2.1 (95% C.I., 1.6-2.7) g/kg bw
Combined = 2.7 (95% C.I., 2.2-3.4) g/kg bw

Pine Oil Blend CSMA 1687 is of **Low acute oral Toxicity** based on the combined LD_{50} (EPA Toxicity Category III).

Clinical observations associated with the treatment began on Day 0 and were not observed after Day 4. The major clinical signs included salivation, lethargy, ataxia, respiratory distress, clear bilateral ocular discharge, and prostration. Additionally, urogenital staining (an indirect effect of treatment) and hypothermia (which is commonly observed in stressed rats when death is imminent) were observed. No significant changes were observed in body weights. Treatment-related effects in the necropsy were limited to those animals that died prematurely. Major

treatment-related necropsy signs included abnormalities of the brain (hemorrhaged or dilated meningeal vessels), kidneys, adrenal, and gastrointestinal tract.

This acute oral toxicity study is classified **ACCEPTABLE (GUIDELINE)**. This study satisfies the guideline requirement for an acute oral toxicity study on the Pine Oil Blend CSMA 1687 (OPPTS 870.1100; OECD 401) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements are provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Pine Oil Blend CSMA 1687

Description:	Clear, pale yellow liquid
Lot/Batch #:	Not reported
Purity:	Not reported
CAS # of TGAI:	Not reported

2. Vehicle and/or positive control: No controls were used in the study

3. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley	
Age/weight at dosing:	Young adult; 200-286 g (both sexes combined)	
Source:	Charles River	
Housing:	Suspended wire-mesh cages	
Diet:	Purina Certified Rodent Chow #5002 provided *ad libitum*; analyses of feed is performed and provided by the manufacturer	
Water:	Tap water from on-site wells provided *ad libitum*; analyses of water is performed twice per year in accordance with S.O.P. No. A-020	
Environmental conditions:	**Temperature:**	21-22°C
	Humidity:	37-60%
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	7 days minimum	

B. STUDY DESIGN and METHODS:

1. In life dates: Start: 2/9/1987 End: 3/3/1987

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1. Doses were based on an initial range-finding assay that tested 1 animal/sex/dose at doses of 750, 1500, 2500, 3500, and 5000 mg/kg. It was reported that the animals in the 3500 and 5000 mg/kg dose groups died. Based on the mortality results, doses of 2183, 2500, and 2863 mg/kg were administered to groups of five male and five female rats. Based on results from these dose groups, one additional group of five females was dosed at 1907 mg/kg and two additional groups of five males each were dosed at 3278 and 3753 mg/kg.

Following an 18-20 hour fast, rats were given a single dose of Pine Oil Blend CSMA 1687 by gastric intubation with snub-tipped oral dosing needles affixed to a 1 or 3 mL syringe. Individual animal doses were based on body weights taken prior to dosing, and the dose volume was determined by dividing the dosage level in g/kg by the density of the test substance.

Animals were observed for mortality and clinical observations 1, 2.5, and 4 hours after dosing, then once daily for 14 days. Body weights were measured on days -1, 0, 7, 14, and at death. Survivors were sacrificed and a necropsy in which the major organs of the cranial, thoracic, and abdominal cavities were examined was performed.

TABLE 1. Doses, mortality/animals treated

Dose Groups	Dose (mg/kg bw)	Males	Females	Combined
1	1907	---	2/5	2/5
2	2183	0/5	3/5	3/10
3	2500	1/5	3/5	4/10
4	2863	1/5	4/5	5/10
5	3278	2/5	---	2/5
6	3753	5/5	---	5/5

3. Statistics: The oral LD_{50} was calculated using the Litchfield and Wilcoxon method.

II. RESULTS AND DISCUSSION:

A. MORTALITY: Mortality results are presented in Table 1 above. All deaths occurred on day 0 through day 3, the majority occurring on day 1.

The oral LD_{50} (95% C.I.) for males is 3.1 (2.7-3.6) g/kg
females is 2.1 (1.6-2.7) g/kg
combined is 2.7 (2.2-3.4) g/kg

The slope (95% C.I.) for males is 1.18 (1.03-1.34)
females is 1.52 (0.69-3.34)
combined is 1.66 (0.99-2.80)

B. CLINICAL OBSERVATIONS: The study authors reported that the main effects due to the test article were salivation, evidence of salivation (dried, brown staining around the mouth), lethargy, ataxia, respiratory distress (bradypnea and/or respiratory rales), clear bilateral ocular discharge, and prostration. The incidence rates of clinical effects are presented in Table 2 below. Urogenital staining may have been indirectly related to the test article treatment. Prostration was noted in approximately one-half (21/45) of the animals, 15 of which died prematurely. Hypothermia was observed in 14/45 animals and was reportedly due to the imminent death of severely stressed animals. Piloerection, dried, red material around the eyes, and hair loss also occurred but at low incidence rates. Clinical effects were observed across all dose levels tested in the study. Aside from one female with hair loss at the base of the tail on days 6-14, all surviving animals were considered normal by day 5 of the study.

TABLE 2. Clinical Observations

Clinical Observations	Dose Groups (mg/kg) - Males					Dose Groups (mg/kg) - Females				Total No.
	2183	2500	2863	3278	3753	1907	2183	2500	2863	
Number of Animals Examined	5	5	5	5	5	5	5	5	5	45
Salivation	1	4	5	2	3	4	5	5	5	34
Evidence of Salivation	1	5	1	4	5	2	1	2	0	21
Ataxia	5	4	5	4	1	4	4	3	5	35
Lethargy	4	5	5	4	2	4	3	4	5	36
Prostrate	0	2	1	1	4	4	3	4	2	21
Respiratory Distress	2	2	4	2	3	4	4	4	5	30
Bradypnea	1	2	4	1	3	4	4	4	5	28
Respiratory rales	2	0	0	1	1	0	1	0	0	5
Clear Ocular Discharge	0	3	3	1	3	3	3	5	3	24
Hypothermia	0	0	4	0	0	0	3	2	5	14
Urogenital Staining	3	1	5	3	1	5	3	3	2	26
Wet, yellow	2	1	5	3	1	4	3	3	2	24
Dried, yellow	3	1	1	1	3	1	2	1	0	12
Wet, red	0	0	0	0	0	0	0	0	1	1

C. **BODY WEIGHT:** No significant body weight variations were noted.

D. NECROPSY: The most common effects found in the animals that died prematurely were hemorrhage and congestion of the brain (meningeal vessels). Other abnormalities in animals that died prematurely included the following: kidney abnormalities (pale, cysts, reddened, cortico-medullary junction dark red) in 15/21 animals; abnormalities of the adrenal glands (reddened and/or enlarged), intestine (reddened contents), and lungs (bright or dark red) in approximately one-half of the 21 animals; stomach abnormalities (reddened mucosa, black areas, yellow contents) in 8/21 animals (7 males and 1 female); abnormalities of the liver (pale, dark purple, mottled, soft), thymus (reddened), and urinary bladder (distended with red fluid) in 4 or 5 of the 21 animals. Alterations in the lungs and thymus were not considered treatment-related, but instead were considered to be typical agonal changes. Urinary bladder distention was observed only in females. Additionally, single findings of thick yellow tracheal contents, cystic ovary, and

dark, red spleen also were observed in the animals that died prematurely.

No significant changes were noted in the animals that were sacrificed at the end of the study period.

E. REVIEWER'S CONCLUSIONS: The observed salivation was probably due to the route of administration rather than the test article because the effect was noted only on day 0 and did not persist. The assessment of the LD_{50} appears to be reasonable based on the mortality data. Due to the lower LD_{50} value, females appear to be more sensitive to the test article than males. Overall the study was well performed and the protocol was appropriate for this type of assay.

F. DEFICIENCIES: Some minor study design and reporting deficiencies are:

- The study author only provided the summary data for the necropsy findings.
- Purity and lot number were not reported in the study because they were not provided by the supplier.

These deficiencies are not expected to effect the outcome of the study.

G. STUDY CLASSIFICATION: This acute oral toxicity study is classified **ACCEPTABLE (GUIDELINE)**. This study satisfies the guideline requirement for an acute oral toxicity study on the Pine Oil Blend CSMA 1687 (OPPTS 870.1100; OECD 401) in the rat.